Synthesis of cyclohexenyl nucleoside triphosphates.

Cyclohexenyl-guanine nucleoside triphosphate and cyclohexenyl-adenine nucleoside triphosphate were synthesized according to the one pot synthesis described by Ludwig (1).

A cold mixture of trimethylphosphate (300 μl) and phosphorous oxychloride (15 μl, 1.5 eq) was stirred under argon in a dried flask for 5 minutes. This mixture was added under argon to the dried starting material (the cyclohexenyl nucleoside, 0.1 mmol). The reaction was stirred under argon for 2 hours, on ice. After 2 hours a mixture of tetra(tributylammonium)pyrophoshate (0.5 mmol, 5 eq) in 1 ml dry DMF and 120 μl tributylamine was added under argon and with vigorous stirring (the reaction was kept on ice). The reaction was quenched by transferring the reaction mixture to a cold solution of TEAB 1M (pH 7.5) while stirring. After 40 minutes the solution was concentrated to dryness and purified by HPLC using a gradient of TEAB 0.5 M (from 0% TEAB to 100% TEAB in 30' and 10' TEAB 100%). As indicated by mass spectrometry and NMR the product were the desired nucleoside triphosphates.